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EFFECT OF UREMIC TOXINS IN VARIOUS CLINICAL CONDITIONS

Protein-bound uremic solutes: The forgotten toxins

RAYMOND VANHOLDER, RITA DE SMET, and NORBERT LAMEIRE

Nephrology Section, Department of Internal Medicine, Ghent University Hospital, Ghent, Belgium

Protein-bound uremic solutes: The forgotten toxins. The present concept of dialysis focuses mainly on the removal of small water-soluble compounds, and also, the currently applied kinetic parameters of dialysis adequacy are based on the behavior of water-soluble compounds. Nevertheless, many of the currently known biological effects in uremia are attributable to compounds with different physicochemical characteristics, and among these, protein-bound solutes play an important role. In this article, we review the characteristics and consequences of changes in protein binding in uremia, as well as the toxicity of the protein-bound uremic solutes 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid (CMPF), indoxyl sulfate, hippuric acid, homocysteine, and p-cresol. Starting from the example of p-cresol, we then summarize the impact of protein-binding on dialytic removal, whereby it is concluded that this removal is largely hampered by this protein-binding compared with that of classic markers such as urea and creatinine. Alternative removal strategies, such as strategies to modify intestinal generation or absorption, are considered.

Uremic retention solutes can be subdivided in three different categories according to their physicochemical characteristics and their subsequent behavior during dialysis: (*1*) the small, water-soluble, non-protein–bound compounds (molecular weight of <300 D; for example, urea), (*2*) the larger middle molecules, mainly peptides, with a molecular weight of between 300 D and 12,000 D, and (*3*) the small protein-bound compounds.

The present concept of dialysis focuses mainly on the removal of small water-soluble compounds, since many dialyzer membranes are partially or entirely hydrophilic, whereas by definition, dialysate is a hydrophilic milieu as well. Not only the concept of dialysis, but also the currently applied kinetic parameters of dialysis adequacy are based on the behavior of the water-soluble compounds urea and creatinine. The removal of the middle molecules or the protein-bound solutes with any of the current strategies is rarely as efficient as that of urea or other small, water-soluble compounds.

The protein-bound compounds are at least partially hydrophobic. Protein binding and hydrophobicity are remarkably correlated [1]. It is therefore conceivable that water-soluble markers are not representative in their dialytic behavior for those protein-bound hydrophobic/lipophilic compounds. In this article, the clinical, biochemical, and kinetic aspects of protein binding in uremia are reviewed.

Key words: uremic toxicity, dialysis, solute removal, p-cresol, CMPF, indoxyl sulfate, homocysteine, hippuric acid.

PROTEIN BINDING OF DRUGS

The first studies regarding protein binding in uremia have focused on drugs. For organic acid drugs, a highly significant negative correlation between protein binding and serum creatinine has been described [2]. The responsible competitors were supposed to be protein-bound organic acids. Studies aiming at analytical identification of compounds in fractions of uremic ultrafiltrate made it possible to characterize hippuric acid as a single solute that inhibited protein binding of both theophilline and phenytoin [2].

The latter study was, however, compromised by several biasing factors: (*1*) high-performance liquid chromatography (HPLC) studies had been undertaken on uremic ultrafiltrate, wherein by definition the molecules with the strongest protein binding are present at extremely low concentrations, and (*2*) the HPLC procedure in that specific study was stopped at a gradient of 46% formate/54% methanol, that is, when most of the lipophilic (and hence protein-bound) solutes had not yet eluted from the chromatographic column.

In later studies, the HPLC procedure was therefore extended up to 100% methanol, and deproteinization methods were applied, which increased the release of protein-bound solutes from their binding sites [3]. When more protein-bound ligands were released from their binding sites by this approach, lyophilisate of the resulting solutions induced a more substantial inhibition in normal serum of theophilline protein binding than lyophilisate of ultrafiltrate [4]. Subsequent spiking experiments made it possible to identify several new compounds: indoxyl sulfate, tryptophan, 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid (CMPF), and indole-3-acetic acid [4]. All of these compounds elute to the right of the HPLC, that is, in the lipophilic range (Fig. 1).

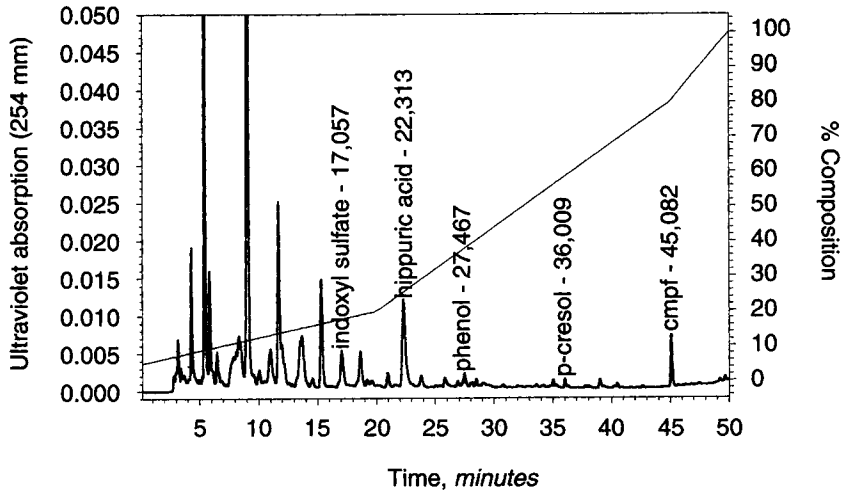

Fig. 1. High-pressure liquid chromatography (HPLC) elution pattern of several protein-bound compounds. Analysis of a deproteinized uremic serum sample. The name of the peaks together with their retention times in minutes are mentioned. Left vertical axis, ultraviolet absorption (AU); right vertical axis, percentage of methanol. This gradient (from 0 to 100% for a chromatography time of 50 minutes) is indicated by the full line. The slope of the gradient is modified twice, according to the density of peaks in each specific elution zone.

UREMIC TOXICITY OF PROTEIN-BOUND SOLUTES

Protein binding of drugs is, of course, not the most typical example of a uremic toxic effect, because of the negligible impact on the clinical status of the uremic patient. The question arises of whether the protein-bound compounds affecting drug protein binding simultaneously affect biological functions relevant to the uremic syndrome. Fractions from HPLC eluate in the lipophilic range have been found to inhibit key biochemical functions, such as the metabolic clearance of calcitriol [5] or the activity of nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase, an enzyme present in phagocytic cells and involved in the destruction of invading bacteria by production of free radicals [6]. The responsible compounds could not, however, be identified in these studies.

In what follows, the main toxic side-effects of specific protein-bound uremic solutes (Fig. 2) are reviewed. Most of these solutes are the same ones as those influencing drug protein binding.

3-Carboxy-4-methyl-5-propyl-2-furanpropionic acid

3-Carboxy-4-methyl-5-propyl-2-furanpropionic acid (CMPF) is one of the urofuranic fatty acids, which are strongly lipophilic structures. It inhibits the renal uptake of para-aminohippuric acid (PAH) in rat kidney cortical slices [7] and causes a decrease in renal excretion of various drugs or of their metabolites and of endogenously produced organic acids, which are removed via the PAH pathway. In vivo, renal CMPF clearance in the rat is inhibited by PAH and probenecid, the latter, however, at a molar dose exceeding at least fivefold that of CMPF [8]. CMPF inhibits hepatic glutathione-S-transferase [9], deiodination of T4 by cultured hepatocytes [10], and adenosine diphosphate (ADP)-stimulated oxidation of nicotinamide adenine dinucleotide (NADH)-linked substrates in isolated mitochondria [11]. Costigan, O'Callaghan, and Lindup demonstrated a correlation between neurologic abnormalities and plasma concentration of CMPF in renal failure patients [12].

Indoxyl sulfate

Indoxyl sulfate is metabolized by the liver from indole, which is produced by the intestinal flora as a metabolite of tryptophan. It inhibits the active tubular secretion of organic acid compounds [13], as well as the deiodination of thyroxine 4 (T4) by cultured hepatocytes [10].

It is known that uremic retention solutes induce glomerular sclerosis [14] and that their removal by peritoneal dialysis or by oral sorbent administration retards the progression of intact nephron loss. Indoxyl sulfate might be one of the possible candidates for the enhancement of glomerular sclerosis. The oral administration of indole or of indoxyl sulfate to uremic rats causes a faster progression of glomerular sclerosis and of renal failure [15].

Hippuric acid

Hippuric acid, as a protein-bound compound, may enhance drug toxicity and toxicity of other protein-bound uremic solutes by competition for protein binding [2, 4]. It also inhibits tubular organic acid secretion, in analogy to indoxyl sulfate and CMPF [13]. The organic acid transport system is inhibited in an identical way in other cell systems. Hippuric acid has been related to insulin resistance and glucose intolerance [16].

Homocysteine

Homocysteine (Hcy) is a sulfur-containing amino acid produced by the demethylation of methionine. Its retention results in the cellular accumulation of S-adenosyl Hcy (AdoHcy), an extremely toxic compound that competes with S-adenosyl-methionine (AdoMet) and inhib-

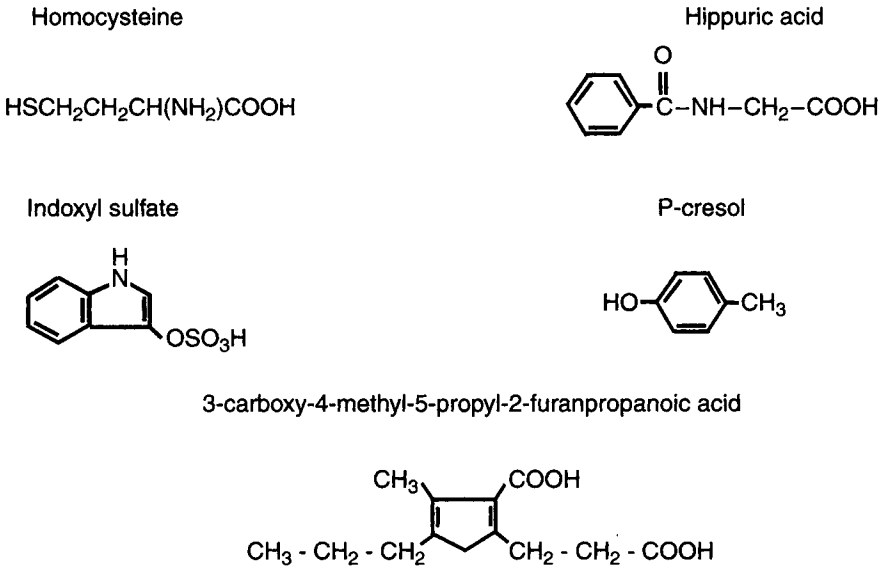

Fig. 2. Chemical structure of protein-bound uremic solutes having an impact on biological functions related to the uremic syndrome.

its methyltransferases [17]. Moderate hyperhomocysteinemia is an independent risk factor for cardiovascular disease in the general population [18].

Patients with chronic renal failure have total serum Hcy levels that are twofold to fourfold above normal. Hyperhomocysteinemia is the most prevalent cardiovascular risk factor in end-stage renal disease (ESRD) [19], and is present as well at increased concentrations in kidney transplant recipients with cardiovascular disease [20]. However, its serum concentration not only depends on the degree of kidney failure, but also on nutritional intake (for example, of methionine), vitamin status (for example, of folate), genetic factors, and renal metabolization.

Homocysteine increases the proliferation of vascular smooth muscle cells, one of the most prominent hallmarks of atherosclerosis [21]. The administration of excess quantities of the Hcy precursor methionine to rats induces atherosclerosis-like alterations in the aorta [22]. Hcy also disrupts several vessel wall-related anticoagulant functions, resulting in enhanced thrombogenicity [23].

p-CRESOL AS A PROTOTYPE

The study of kinetics of protein-bound compounds in normal renal function, uremia, and dialysis necessitates the evaluation of a molecule with substantial protein binding, proven toxicity, and that can reliably be determined with a currently available method. p-Cresol fulfills these criteria [24, 25].

Biochemical effects of p-cresol

Our interest in this molecule has been raised by the finding of an inhibitory effect on leukocyte response following phagocytosis [26]. In addition, p-Cresol also increased the in vitro toxicity of aluminum in hepatocytes and neuroblastoma cells [27]. Additional studies also revealed an inhibitory effect on monocyte synthesis of platelet-activating factor (PAF) [28]. Several other biological effects of this molecule have recently been reviewed [29].

Intradialytic kinetics of p-Cresol

p-Cresol and other protein-bound molecules show an intradialytic removal pattern that differs substantially from that of the current water-soluble markers, urea, and creatinine [30]. The procentual removal of indoxyl sulfate, indole-acetic acid, CMPF, and p-Cresol is significantly less important than that of urea and creatinine. Only the removal of hippuric acid is not significantly different, but this compound is not strongly protein bound. There are no differences in the removal of p-Cresol by high-flux compared with low-flux membranes. Although removal of urea and creatinine intermutually correlate, there is no correlation with the removal of the protein-bound compounds. Urea and creatinine removal are thus not representative of the intradialytic behavior of protein-bound molecules.

Role of the intestine

Another potential strategy to lower plasma levels of p-Cresol and/or other protein-bound uremic toxins is to decrease intestinal absorption. P-cresol is produced from the amino acids phenylalanine and tyrosine by intestinal bacteria. Oral administration of the intestinal adsorbant AST-120 to uremic rats results in significantly lower plasmatic levels of phenol and p-Cresol [31]. Remarkably, the same adsorbent also slows down the progression

of renal failure by attenuating glomerulosclerosis in 5/6 nephrectomized rats [14].

The important role of the intestine in the production of p-Cresol is further emphasized by the influence of dietary protein supplementation on the formation of bacterial metabolites in the colon [32], the influence of antibiotics affecting intestinal flora on the excretion of volatile phenolic and aromatic bacterial metabolites [33], and the modification of the production of phenolic compounds by the shift to an uncooked vegan diet [34].

CONCLUSIONS

Lipophilic and/or protein-bound uremic solutes have an important biological impact. Their kinetics and dialytic removal pattern are different from those of the classic uremic markers such as urea and creatinine. The removal of the protein-bound compounds with conventional hemodialytic strategies, even high-flux dialysis, is unsatisfactory. The role of the intestine in their production has often been neglected.

Virtually all middle molecules (β_2-microglobulin, various peptides, and leptin) and all protein-bound molecules, as well as some of the small hydrophilic molecules (xanthine, hypoxanthine, and phosphorus) [35], differ in their dialytic behavior from urea. Most of these molecules are responsible for biochemical or biological changes and take part in the uremic syndrome [36]. In contrast, many of the molecules with a kinetic behavior comparable to urea (creatinine, pseudouridine) exert no such strong biological activities. The validity of water-soluble compounds as representative markers of uremic toxicity and dialytic removal should therefore be questioned.

The application of alternative removal strategies, for example, adsorption on charcoal and resins, might be considered. In vitro studies on uremic ultrafiltrate demonstrated that adsorption of protein-bound uremic solutes was highly efficient [37]. It is, however, uncertain whether a similar efficacy can be expected in vivo as well.

Reprint requests to Dr. Raymond Vanholder, Nephrology Section, Department of Internal Medicine, Ghent University Hospital, De Pintelaan 185, B9000, Ghent, Belgium.
E-mail: raymond.vanholder@rug.ac.be

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